Short communication

A simple method to determine transpiration efficiency in sorghum[☆]

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Abstract

Sorghum [*Sorghum bicolor* (L.) Moench] is a C4 cereal grain crop grown primarily in arid and semi-arid regions in the world with limited or no irrigation. Sorghum production fluctuates and largely depends on the amount and distribution of rainfall. Transpiration efficiency (TE), the biomass produced per unit water transpired, could be a potential trait to improve sorghum yield in areas where irrigation is limited. We have developed a mini-lysimetric method that directly measures whole plant TE in sorghum during an early vegetative stage under greenhouse conditions. The method was evaluated with 11 inbred lines and three hybrids under two greenhouse environments. In general, TE determined with the gravimetric method was higher under lower vapor pressure deficit conditions; however, similar rankings in TE were obtained across the experiments. The method described in this report offers a simple, high-throughput, and affordable way to determine the integrated TE in sorghum at an early vegetative stage.
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Keywords: Transpiration efficiency; Sorghum; Genetic variation

1. Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is a C4 plant well adapted to semi-arid environments. In the Southern Plains of the USA and many other areas of the world, sorghum is usually cultivated under rain-fed conditions. The yield of sorghum grown under such conditions strongly depends on the rainfall during the growing season and subsoil moisture stored from prior season rains. Achieving greater yield per unit rainfall is one of the most important challenges in dryland agriculture. Enhancing transpiration efficiency (TE), the dry biomass produced (g) per unit water transpired (kg), may be an important means of improving sorghum yield in semi-arid and arid regions. A high TE trait could either enable plants to produce more biomass from the same amount of available soil water, delay the onset of detrimental water deficit stress by conserving soil water, or a combination of both. Wheat cultivars selected for low carbon isotope discrimination ratio ($\Delta^{13}C$), a reliable surrogate for TE in C3 plants, were shown to have significantly higher grain yields in low rainfall environments (Rebetzke et al., 2002). However, little work has been done to improve TE in sorghum.

It is known that sorghum varies in TE, as determined by instantaneous gas exchange methods and lysimeters (Peng and Krieg, 1992; Mortlock and Hammer, 1999). Due to the complexity and labor requirements of these methods, they have not been commonly used to select high TE lines for sorghum germplasm improvement. The $\Delta^{13}C$ discrimination method developed by Farquhar et al. (1982), which has been widely used in C3 plants to estimate TE, is not applicable to sorghum, a C4 plant (Henderson et al., 1998; Lambrides et al., 2004). To effectively improve TE in sorghum, a high-throughput and cost-effective method is needed to screen the genetic variation of TE from a large germplasm collection. Here, we report a simple and affordable mini-lysimetric method that allows the high-throughput determination of whole plant level TE in sorghum during an early vegetative stage.

2. Materials and methods

Whole plant level TE was determined gravimetrically in 2-liter plastic pots with a diameter of 14 cm and height of 16 cm as illustrated in Fig. 1. The pots were filled with Sunshine #1 potting mix (Sun Gro Horticulture Inc., Bellevue, WA) and watered with $0.5\times$ Miracle-Gro (Scotts Miracle-Gro Products, Inc., Marysville, OH) until dripping from the bottom. Several
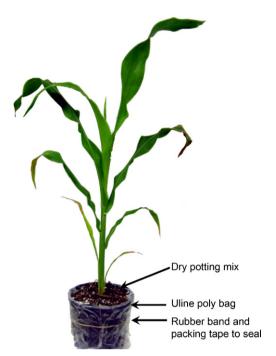

Fig. 1. Illustration of the mini-lysimetric determination of whole plant level transpiration efficiency (TE).

soil potting mixes were tested; Sunshine #1 was chosen for its high water-holding capacity. When saturated, each pot can hold approximately 1 kg of available water, which was sufficient to support the growth of sorghum seedlings to the eight-leaf stage. Three seeds were planted per pot and thinned to one plant at 7 d after emergence. The pots were then covered from both ends with 2 mil poly bags purchased from Uline (Waukegan, IL). A small slit was made in the top bag to allow the sorghum plant to grow through. The slit was sealed with Scotch packing tape and covered with a layer of dry potting mix to prevent water loss through the opening in the top bag. The poly bags were tightly fixed onto the pots with an elastic band. The pots were bar-coded, and the initial weight was taken automatically with Mettler Toledo's Balance Link program and SB12001 balance (Columbus, OH). The plastic bagging effectively minimized

the water loss from the soil surface. During the entire experimental cycle, control pots without plants lost only negligible amounts of water (<20 g), which was not included in the calculation of TE.

Two tests were performed to determine if the plastic bags had adverse effects on sorghum growth and development (data not shown). First, entire sorghum seedlings and the pots were completely sealed in plastic bags; sorghum seedlings continued to grow well in such sealed conditions, indicating that the bags adequately allow air exchange with the ambient environment. Second, the growth of sorghum seedlings in open pots was compared with seedlings in sealed pots; no difference in growth was observed until the water became limiting in the open pots. Thus, the plastic bags used in this experiment were unlikely to impose a hypoxic stress to the plants.

Plants were harvested 4 weeks after planting (the leaf collar of the eighth leaf became visible). At this stage, no obvious leaf wilting was observed. The final pot weight (including roots) was taken at the end of the experiment. Water used was calculated by subtracting the final pot weight from the initial weight. Roots were collected by washing the potting mix core on a wire mesh (4 mesh/in.). Dry weight measurements of roots and shoots were taken after a minimum of 72 h of drying at 80 °C when the samples reached a constant weight. Shoot TE was calculated by dividing the dry biomass of above ground tissue by the amount of water transpired; total TE was calculated by dividing the total dry biomass (above ground biomass plus root) by the amount of water used.

In order to be useful as a screening or selection tool, a method should be cost-effective, reproducible, and high-throughput way to determine TE. To ensure that the screen is widely applicable, it should be possible to conduct the experiments under conditions where environmental parameters cannot be controlled rigorously. This experiment was completed in approximately one month with minimum hands-on time, and the material cost for each sample was less than $0.50. The validity of this method as a screening tool was evaluated with 11 inbred lines and three hybrids that represented a wide range of variation in sorghum, including but not limited to drought tolerance (Table 1). Two greenhouses

Table 1
Pedigrees and characteristics of the 14 lines in the WUE study

Name	Pedigree or origin	Drought tolerance
BTx642	(BTx406*IS12555(SC35)$_{F3}$)*IS12555	Stay green
Tx7078	Old U. S. landrace, pedigree unknown	Excellent pre-flowering drought tolerance
BTx623	(BTx3197*SC170-6-4)-7-3-1-3-2-1	Unknown
SC 170-6-17	SC 170/3/4-dwarf Martin B line/SC 170/2/SC 170*2/4/SC 170	Unknown
Ajabsido	Race: Caudatum; working group: Feterita; origin: Sudan	Excellent pre-flowering drought tolerance
El Mota	Race: Caudatum origin: Niger	Excellent pre-flowering drought tolerance
Liang Tang Ai	Race: Bicolor; working group: Kaoliang; origin: China	Unknown
CSM 63	Race: Guinea; working group: guineense; origin: Mali	Fair pre- and post-flowering drought tolerance
CSM 205C	Race: Guinea; working group: guineense; origin: Mali	Unknown
IS 3620C	SC 303/3/4-dwarf Martin B line/SC 303/2/SC 303*2/4/SC 303; origin: Nigeria	Unknown
Tx7000	Old U. S. landrace Milo × Kafir selection, released in 1941	Excellent pre-flowering drought tolerance
Pioneer85G85	Unknown	Unknown
Pioneer86G71	Unknown	Unknown
Pioneer8500	Unknown	Unknown

with different temperature and relative humidity ranges were used to test the reproducibility of the method. The plants were grown under natural irradiation. Temperature and relative humidity were monitored by a Hobo Pro RH/Temp series data logger that records both relative humidity and temperature (Bourne, MA). In experiment 1, the greenhouse daily minimum, average, and maximum temperatures were 20, 24, and 28 °C, respectively, and the average vapor pressure deficit (VPD) was 2.3 kPa. In experiment 2, the daily minimum, average, and maximum temperatures were 19, 22, and 24 °C, respectively, and the average vapor pressure deficit was 1.4 kPa.

Means separation was performed on the lines using the GLIMMIX procedure in SAS (version 9.1.3) for the shoot and total TE variables. The data was analyzed as a randomized complete block design with block as a random effect. The LSMEANS option was used to calculate Tukey's HSD groupings of the lines. The means for dependent variables were rank ordered within each experimental location using the RANK procedure in SAS. The rank variables were analyzed by Wilcoxon Signed Rank (non-parametric paired t test) in the UNIVARIATE procedure in SAS.

3. Results and discussion

3.1. Effect of genotype and environment on transpiration efficiency

The variation in TE among the 11 inbred lines and three commercial hybrids was analyzed separately for each greenhouse experiment. The effect of line on TE was significant ($p < 0.0001$) under both conditions (Table 2). Environmental factors, especially vapor pressure deficit, have dramatic effects on TE. Instantaneous TE, the ratio of carbon dioxide assimilation to transpiration at the leaf level, is known to be inversely proportional to VPD (Tanner and Sinclair, 1983). Our

Table 2
Average transpiration efficiency (TE) based on shoot dry biomass and total biomass of 14 sorghum lines

Genotypes	Shoot TE (g/kg)		Total TE (g/kg)	
	EXP 1	EXP 2	EXP 1	EXP 2
Liang Tang Ai	6.9 a	7.7 a	8.3 ab	8.8 a
SC170-6-17	6.6 ab	7.3 ab	8.4 a	8.8 a
BTx623	6.5 ab	7.3 abc	8.1 ab	8.5 ab
Pioneer85G85	6.4 ab	7.3 abc	8.5 a	8.7 a
B35/BTx642	6.2 abc	7.1 abc	8.3 ab	8.5 ab
El Mota	6.1 bcd	7.0 abc	7.8 abc	8.3 ab
Pioneer86G71	6.0 bcd	7.0 abc	7.8 abc	8.3 ab
Pioneer8500	5.6 cde	6.7 bcd	7.0 bcd	8.3 abc
IS3620C	5.5 cde	6.5 cde	8.0 ab	8.0 abcd
TX7000	5.5 cde	6.5 cdef	7.1 bcd	7.6 bcde
Ajabsido	5.5 de	5.7 f	7.3 abcd	7.3 de
CSM205C/SC1426C	5.3 e	5.8 ef	7.6 abc	7.4 cde
CMS63	5.2 e	6.7 bc	6.7 d	8.1 abcd
TX7078	5.0 e	6.0 def	6.3 d	6.9 e
Average	5.9	6.7	7.7	8.1

Tukey–Kramer grouping of the inbred and hybrid lines using least square means option in GLIMMIX procedure (SAS version 9.1.3). LSMEANS estimates with the same letter are not significantly different.

measurements of integrated TE, as determined by the ratio of biomass produced per unit water transpired, were also affected by VPD. In experiment 1 (high VPD), all lines displayed lower TE than in experiment 2 (low VPD) (Table 2). The average TE based on shoot biomass for the 14 lines was 5.9 and 6.7 g/kg for experiments 1 and 2, respectively. The average TE based on total biomass was 7.7 and 8.1 g/kg, respectively. This result is consistent with previous reports that plants have lower TE under higher VPD (Tanner and Sinclair, 1983).

3.2. Effect of biomass accumulation and water use on transpiration efficiency

At the leaf level, improved TE can result from either decreased stomatal conductance or increased photosynthetic capacity (Condon et al., 2002). Similarly, integrated TE determined by the gravimetric method can be improved either through decreased transpiration or increased biomass production integrated over a period of time. To determine which components contributed to TE in this study, a correlation analysis was conducted between TE and biomass accumulation and between TE and total water use. Both shoot biomass and total biomass were positively correlated with TE at $r = 0.48$ and $r = 0.39$, respectively. No correlation was found between water use and TE based either on shoot or total biomass ($r = 0.03$ and $r = 0.09$). We also examined whether seed weight contributed to plant biomass, and hence, TE. Average seed weight for the lines used in the experiments ranged from 18 mg (IS3620C) to 36 mg (SC170-6-17). However, no correlation was found between seed size and TE based on either shoot biomass or total biomass ($r = -0.15$ and $r = -0.22$, respectively). Thus, the sorghum lines selected with increased integrated TE were at least partly associated with higher biomass accumulation rather than reduced transpiration. It is possible that selection for high TE lines with increased biomass accumulation may reduce the chance of selecting plants with an undesired reduction in growth, which is often associated with the lines chosen based on the $\Delta^{13}C$ discrimination method (Condon et al., 2002; Impa et al., 2005).

3.3. Reproducibility of transpiration efficiency determined by the gravimetric method

The Wilcoxon Signed Rank test (non-parametric paired t test) was used to compare the reproducibility of the method within the two environments. The average shoot and total TE obtained was ranked for experiment 1 (high VPD) and experiment 2 (low VPD). The ranks were then analyzed to see if they were different. The p-value for the rank comparison of experiments 1 and 2 shoot TE was 0.98; the p-value for total TE was 0.90. This result indicated that no difference in shoot or total TE rank order was observed between the two experiments. Due to difficulties associated with collecting root biomass, initial screens can be conducted using shoot dry biomass only. Subsequent reconfirmations of potential lines should include TE based on both shoot biomass and total biomass.

3.4. Transpiration efficiency and drought tolerance

The TE values for the 14 sorghum lines were compared to the drought tolerance phenotypes previously reported; no apparent relations were found between TE and pre- or post-flowering drought tolerance. This indicates that the mechanisms controlling TE are distinctive from these controlling drought tolerance. This also suggests that it may be possible to stack high TE traits with drought tolerance to improve sorghum yield under water-limited conditions.

4. Conclusion

We report a high-throughput, cost-effective method to determine TE in sorghum. Genetic variation in TE was detected in 11 inbred lines and three hybrids. Although the environment strongly modulates TE, similar rankings in TE were observed under two greenhouse conditions. The reproducibility in the ranking of TE indicates that integrated TE may be a trait that is worth exploiting to enhance sorghum yield under water-limited conditions. Given the reproducibility and simplicity, the method reported here may provide a useful technique to select high TE lines in sorghum.

Acknowledgements

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